

$\text{NH}_3^+ \cdots \text{S}$ interaction. Such an intramolecular sulphur-nitrogen interaction has been postulated in Doherty's theory of radioprotection⁶ and has also been observed in the crystal structure of another radioprotectant β -mercapto ethylamine hydrochloride⁷.

The crystal structure consists of layers of cystamine molecules stacked parallel to the *ab*-plane. Both sides of each layer are lined with positively charged amino groups and adjacent layers are connected through chloride ions. The interface between layers involves ionic interactions between amino groups and the chloride ions and $\text{NH} \cdots \text{Cl}$ hydrogen bonds. The nitrogen atom N(1) is surrounded by three chloride ions and the nitrogen atom N(2) by four chloride ions, two of which appear to be involved in a bifurcated hydrogen bond.

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COLORIMETRIC DETERMINATION OF MICROGRAM QUANTITIES OF ISONIAZID

SIMPLE colorimetric method of estimation of isoniazid is reported. In alkaline medium isoniazid forms an intense coloured species with catechol, levo-3 (3, 4-dihydroxyphenyl)-2 methylalanine (methyldopa) and *p*-aminophenol (PAP). Beer's law is obeyed in the concentration range of 1 to 11 $\mu\text{g./ml.}$ of isoniazid with catechol, 2.5 to 70 $\mu\text{g./ml.}$ of isoniazid with methyldopa and 10 to 80 $\mu\text{g./ml.}$ of isoniazid with PAP.

Isonicotinic acid hydrazide commercially known as isoniazid is an antitubercular drug. The official iodometric method¹ suffers from interference of vitamin 'C'. The colorimetric methods are with *p*-dimethylaminobenzaldehyde²⁻⁵, 1, 2-naphthoquinone-4-sulphonic acid⁶⁻⁹, 1-chloro-2, 4-dinitrobenzene², Na-pentacyanamine ferrate¹⁰, phosphomolybdic acid⁵, 3, 4-dinitrobenzoic acid¹¹, Vanadium¹², etc. Many commercial samples of isoniazid are formulated with vitamin 'C' and hence a method for colorimetric estimation of isoniazid in presence of vitamin 'C' has been developed using catechol, methyldopa and PAP.

Experimental

Catechol (0.04% aqueous), 0.1% methyldopa in 0.1 M HCl, 0.04% PAP (ethanolic) were used as reagents.

Standard isoniazid solution of the concentration of 100 $\mu\text{g./ml.}$ was prepared in water.

Sample solution (100 $\mu\text{g./ml.}$) was also prepared in water. The absorbance readings were noted on C.Z. colorimeter equipped with matched glass cells.

Assay Procedure

(1) Catechol : 0.5 ml. of each standard and sample were taken separately in 10 ml. graduated test-tubes, 5 ml. of 1.0 M ammonia and 0.4 ml. of the reagent were added. The volume was adjusted to the mark with water and after 5 minutes the absorbance was noted at 485 nm against the reagent blank.

(2) Methyldopa : 2 ml of each standard and sample were taken separately in 10 ml graduated test-tubes, 5 ml of 1.0 M ammonia and 0.5 ml of the reagent were added. The volume was adjusted to the mark with water and after 5 minutes the absorbance readings were noted at 485 nm against the reagent blank.

(3) PAP : 3 ml of each standard and sample were taken separately in 10 ml graduated test-tubes, 1.0 ml of 0.1 N NaOH and 0.2 ml. of the reagent were added. The volume was adjusted to the mark with water and the absorbance readings were taken after 15 to 20 minutes at 420 nm against the reagent blank.

Table I summarises the experimental conditions of the estimation of isoniazid using catechol, methyldopa and PAP as reagents.

Table II gives the comparative data of the application of the proposed methods in the estimation of isoniazid in the commercial products.

Discussion

The proposed methods are very simple and less time consuming. The results are reproducible and compare satisfactorily with those obtained from the official method. Interference due to common exci-

TABLE I

Reagent	Volume of reagent	Medium	λ_{max} nm	Stability	Molar absorptivity (lit./g. moles cm.)	Colour
Catechol.	0.4 ml. of 0.04% (aqueous)	5 ml. of 1 M NH ₄ OH	485	After 5 minutes	12.5 × 10 ³	Orange
Methyldopa	0.5 ml. of 0.1% (-1 M HCl)	5 ml. of 1 M NH ₄ OH	485	After 5 minutes	1.70 × 10 ³	Orange
PAP	0.2 ml. of 0.04% (ethanolic)	1 ml. of 0.1 M NaOH	420	After 15 minutes	0.98 × 10 ³	Yellow

TABLE II

Product	Isoniazid labelled (mg.)	Isoniazid found (mg.)			Percentage recovery			Isoniazid found by official method ^{1,13} (mg)
		Cate.	M.dopa	PAP	Cate.	M.dopa	PAP	
Tab. A	100	99.63	99.8	99.73	99.1	100.2	101.2	99.7
Tab. B	75	74.46	74.8	74.6	98.9	99.2	98.8	74.5
Tab. C	150	149.3	149.5	150.1	99.1	99.3	101.2	150.1
Syrup A	100	100.3	100.6	100.5	99.3	98.6	101.6	100.4
Inject. A	$\frac{100}{5 \text{ ml.}}$	100.2	99.9	100.1	28.7	100.9	101.6	..

Tab. A contains 25 mg. of vitamin 'C'.

Inject. A contains 25 mg./5 ml. of vitamin 'C'.

ipients like starch, talc, cellulose, as well as of vitamin 'C' is absent. The percentage recovery is almost 100.

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